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RESEARCH ARTICLE

The clinical significance of interleukin-6 in heart failure: results from the BIOSTAT-CHF study

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Aims

Inflammation is a central process in the pathophysiology of heart failure (HF), but trials targeting tumour necrosis factor (TNF)-α were largely unsuccessful. Interleukin (IL)-6 is an important inflammatory mediator and might constitute a potential pharmacologic target in HF. However, little is known regarding the association between IL-6 and clinical characteristics, outcomes and other inflammatory biomarkers in HF. We thus aimed to identify and characterize these associations.

Methods and results

Interleukin-6 was measured in 2329 patients [89.4% with a left ventricular ejection fraction (LVEF) $\leq$ 40%] of the BIOSTAT-CHF cohort. The primary outcome was all-cause mortality and HF hospitalization during 2 years, with all-cause, cardiovascular (CV), and non-CV death as secondary outcomes. Approximately half (56%) of all included patients had plasma IL-6 values greater than the previously determined 95th percentile of normal values at baseline. Elevated N-terminal pro-brain natriuretic peptide, procalcitonin and hepcidin, younger age, TNF-α/IL-1-related biomarkers, or having iron deficiency, atrial fibrillation and LVEF > 40% independently predicted elevated IL-6 levels. IL-6 independently predicted the primary outcome [HR (95% confidence interval) per doubling: 1.16 (1.11–1.21), $P < 0.001$], all-cause mortality [1.22 (1.16–1.29), $P < 0.001$] and CV as well as non-CV mortality [1.16 (1.09–1.24), $P < 0.001$; 1.31 (1.18–1.45), $P < 0.001$], but did not improve discrimination in previously published risk models.

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Conclusions In a large, heterogeneous cohort of HF patients, elevated IL-6 levels were found in more than 50% of patients and were associated with iron deficiency, reduced LVEF, atrial fibrillation and poorer clinical outcomes. These findings warrant further investigation of IL-6 as a potential therapeutic target in specific HF subpopulations.

Keywords Interleukin-6 • Heart failure • Inflammation • Anaemia • Adverse events • Procalcitonin

Introduction

Inflammation is a key process in the pathophysiology of heart failure (HF).[1] Increased levels of pro-inflammatory cytokines are associated with worse outcomes and adverse cardiac remodelling in patients with HF.[1] Although some benefit has been observed in small studies testing immunomodulatory agents in HF, larger clinical trials targeting tumour necrosis factor (TNF)-α, including the ATTACH and RENEWAL trials, were largely unsuccessful, with even potential detrimental effects at higher doses of infliximab in ATTACH.[1,2] However, the recent Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) evaluating the effects of interleukin (IL)-1β blockade in patients with previous myocardial infarction and elevated high sensitivity C-reactive protein (hsCRP), demonstrated unprecedented benefits in reduced cardiovascular (CV) risk.[3] The results of CANTOS and the accumulating evidence for the role of inflammation in HF resulted in renewed interest to investigate anti-inflammatory agents in HF.

Interleukin-6 is a cytokine with both pro-inflammatory and anti-inflammatory properties.[4] The significance of IL-6 in CV disease has only recently been fully recognized. A large meta-analysis investigated the Asp358Ala single nucleotide polymorphism (SNP) of the IL-6 receptor.[5] Asp358Ala carriers had a 3.4% reduced risk of coronary artery disease for each gene copy. This suggests a causal role for IL-6 signalling in coronary artery disease. Additionally, IL-6 levels are known to increase with age and IL-6 signalling has been implicated in the pathophysiology of common HF co-morbidities including frailty, anaemia of chronic disease, renal disease and atrial fibrillation (AF).[6–8]

Increased cardiac IL-6 and IL-6 receptor mRNA levels have been associated with worsening haemodynamics in advanced HF.[9] Worsening of HF was also associated with the CG genotype of the 174G/C SNP of the IL-6 promoter as well as circulating levels of IL-6, irrespective of left ventricular ejection fraction (LVEF).[10–12] Additionally, significant associations between IL-6 and HF-associated mortality have previously been described in small HF cohorts.[13,14] As such, IL-6 is of special interest in HF as pharmacological agents targeting IL-6 signalling have already been developed and successfully used in a multitude of (autoimmune) diseases (e.g. tocilizumab, sarilumab, siltuxumab) and have recently been reviewed by Garbers *et al.*[15] Nevertheless, inflammation is a multifaceted disease process involving a number of major mediators and underlying signalling processes. It is therefore of interest to establish how IL-6 is related to other biomarkers in HF. Previous studies were limited by their sample size and did not investigate the association of IL-6 with other such biomarkers. We therefore aimed to address this by investigating the relationship between IL-6 and clinical characteristics, outcomes and other biomarkers in HF.

Methods

Patients

This is a retrospective study of the BIOSTAT-CHF index cohort. The characteristics of this cohort have been described previously.[16] Briefly, BIOSTAT-CHF was a multi-centre, multi-national, observational study composed of an index and a validation cohort; 2516 patients from 69 centres across 11 European countries were included in the index cohort on the basis of worsening signs/symptoms and suboptimal treatment of HF. The primary endpoint was a combined outcome of all-cause mortality and unscheduled hospitalization for HF. Secondary endpoints were HF hospitalizations, all-cause and CV vs. non-CV mortality. Cause-specific outcomes were determined by site investigators and not independently adjudicated.

Laboratory indices

Measured laboratory values in the index cohort included IL-6, N-terminal pro-brain natriuretic peptide (NT-proBNP), CRP, procalcitonin (PCT), troponin T, haemoglobin, erythrocyte mean corpuscular volume, complete leucocyte blood count, iron, hepcidin, ferritin, transferrin, and % transferrin saturation at baseline. The plasma levels of the following TNF-α/IL-1 related biomarkers were also determined: ST2, IL-1 receptor type 1/2 (IL-1RT1/IL-1RT2), TNF receptor 2 (TNFR-2) and TNF receptor superfamily members 10c, 13b and 14 (TNFRSF-10c/-13b/-14). The levels of serum creatinine, as well as the estimated glomerular filtration rate (eGFR) based on the Modification of Diet in Renal Disease formula were determined at baseline and at 9-month follow-up. Plasma levels of biomarkers were determined using sandwich or competitive enzyme-linked immunosorbent assays on a Luminex platform (Singulex Inc., Alere Inc., and Roche Inc.).

Echocardiography measurements and exercise testing

Standard echocardiography measurements were available for the majority of patients and were performed 1–2 months before inclusion in the study. These included among others: left and right ventricular size and function, wall thickness, lateral and septal annulus tissue velocities and atrial diameters. All patients underwent a 6-min walk test (6MWT) at the time of inclusion.

Statistical analysis

Statistical analyses were carried out with STATA v.15 SE and R v.3.2.3. Normally distributed variables are presented as mean (standard deviation), non-normally distributed continuous variables as median (interquartile range), and categorical variables as number (percentage). Baseline characteristics are presented stratified to quartiles of IL-6. Statistical significance was considered for $P \leq 0.05$. Baseline characteristics

across IL-6 quartiles were compared using a one-way analysis of variance (ANOVA), the Kruskal–Wallis test and the Chi-square test, where appropriate. The primary and secondary endpoints were censored at 2 years. Proportionality of hazards was assessed based on standardized Schoenfeld residuals. Cox proportional hazards models were used to test for a multivariable association of IL-6 with outcomes. In a forward stepwise manner, we corrected for clinical confounders including age, sex, body mass index, eGFR, smoking status, alcohol consumption, diabetes mellitus, eGFR < 60 mL/min/1.73 m^2 and history of any of the following: AF, myocardial infarction, stroke, peripheral arterial disease, chronic obstructive pulmonary disease and hypertension, the use of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (ACEi/ARBs), β-adrenoreceptor blockers (BBs), mineralocorticoid receptor antagonists, digoxin, diuretics and loop diuretics.

To investigate whether IL-6 can improve published risk prediction models for this cohort,[16] we tested for an increase in model fit when adding IL-6 to the BIOSTAT-CHF risk score for the combined endpoint and all-cause mortality, using the likelihood ratio test. The net reclassification improvement (NRI), integrated discrimination improvement (IDI) and Harrell's C-statistic were used to assess improvements in reclassification and discrimination. The risk score includes age, HF hospitalization in the last year, peripheral oedema, systolic blood pressure, NT-proBNP, haemoglobin, high-density lipoprotein, sodium and beta-blocker use at baseline for the primary outcome. For mortality alone, the BIOSTAT-CHF risk score includes age, blood urea nitrogen, NT-proBNP, haemoglobin and beta-blocker use at baseline. When investigating the association of IL-6 with CV and non-CV mortality, non-CV and CV mortality respectively were used as competing risks. All-cause mortality was similarly used as competing risk for HF hospitalizations. Clinically significant interactions were investigated for the combined outcome. IL-6 plasma levels were transformed to a $\log_2$ scale to denote a doubling of IL-6 plasma levels per 1-unit change in all regression models. Based on a multivariable logistic regression analysis, we identified the strongest predictors of elevated IL-6 levels.

Results

The recruitment period was 24 months and median follow-up was 21 months. IL-6 plasma levels were measured in 2329 (92.6%) of the 2516 patients. A comparison of baseline characteristics between patients with and without IL-6 measurements is presented in the online supplementary *Table* S1. Baseline characteristics for the index cohort with measured IL-6 are presented in *Table 1*. Mean age in the cohort was 69 ± 12 years, 1716 (74%) patients were male and median IL-6 levels were 5.2 (2.8–10.2) pg/mL, with 1327 (56.9%) of patients having IL-6 values greater than the previously reported 95th percentile of normal values (> 4.45 pg/mL).[17] Patients with higher levels of IL-6 were older, more often had HF with preserved ejection fraction (HFpEF) and had a higher prevalence of anaemia, diabetes mellitus and AF. Additionally, patients with higher IL-6 levels were less likely to be able to perform the 6MWT and had an overall lower distance covered. This remained the case after multivariable corrections for demographics, co-morbidities, medication use and New York Heart Association functional class for both successful completion of the test [OR (95% confidence interval, CI) per doubling of IL-6: 0.75 (0.69–0.80), $P < 0.001$], as well as overall distance covered [B (95% CI) per doubling of IL-6: −27.19 (−31.50 to −22.88), $P < 0.001$]. Patients with higher IL-6 levels had significantly lower mean haemoglobin and iron concentrations, in tandem with moderately lower transferrin levels and noticeable reductions in transferrin saturation. Hepcidin differed significantly between IL-6 quartiles ($P < 0.001$) but was higher in the first and last quartile compared to the intermediate ones. Patients with the highest IL-6 values had higher median hepcidin values compared to those with the lowest IL-6 values. Patients with higher IL-6 levels also had on average lower eGFR and higher levels of NT-proBNP and CRP.

In multivariable logistic regression analysis, having IL-6 levels above the 95th percentile of normal values was independently predicted by the logarithms of NT-proBNP [OR (95% CI): 1.30 (1.14–1.49), $P < 0.001$], PCT [1.31 (1.14–1.51), $P < 0.001$], lower iron levels [0.48 (0.38–0.60), $P < 0.001$] and hepcidin [1.27 (1.14–1.40), $P < 0.001$], as well as having AF [1.35 (1.03–1.77), $P = 0.028$], older age [0.87 (0.76–0.99) per 10 years, $P = 0.032$] and HFpEF [1.63 (1.06–2.50), $P = 0.027$]. Most TNF-α/IL-1 related biomarkers including ST2, IL-1RT2, TNFR-2, TNFRSF-13b, and TNFRSF-14 were also independent predictors of IL-6 levels. A forest plot with all included predictors in multivariable logistic regression is presented in *Figure 1*.

Cox proportional hazards and competing risk survival regression models for the primary and secondary outcomes

Patients in the highest quartile of IL-6 experienced the combined outcome twice as much as those in the lowest quartile at 2-year follow-up (*Figure 2*). After correcting for confounders, IL-6 remained significantly associated with the combined outcome [HR (95% CI): 1.16 (1.11–1.21), $P < 0.001$]. When correcting for the BIOSTAT-CHF risk score, higher levels of IL-6 remained associated with the combined outcome [HR (95% CI): 1.08 (1.03–1.13), $P = 0.001$]. Higher levels of IL-6 were equally associated with higher rates of the mortality alone [HR (95% CI): 1.22 (1.16–1.29), $P < 0.001$] as well as death due to non-CV causes [HR (95% CI): 1.31 (1.18–1.45), $P < 0.001$] and CV causes [HR (95% CI): 1.16 (1.09–1.24), $P < 0.001$]. No significant interactions were identified between IL-6 and relevant covariates, when predicting the combined outcome. All subgroup analyses are presented in the online supplementary *Figure S1*. The cumulative incidence function curves for CV/non-CV mortality and HF rehospitalization are presented in the online supplementary *Figures S2–S4*. The proportion of patients with mortality due to CV and non-CV causes did not differ significantly between the four quartiles of IL-6 ($P = 0.27$). However, IL-6 was not a significant predictor of HF hospitalizations alone.

Interleukin-6 improved the model fit of the BIOSTAT-CHF risk model for the combined outcome (likelihood ratio test: $P = 0.002$); however, this did not lead to significant changes in discrimination [$NRI^{>50}$: −0.4%, $P = 0.610$; IDI: 0.2%, $P = 0.031$; Harrell's C baseline: 0.713 vs. Harrell's C IL-6: 0.715]. Similar findings were identified for all-cause mortality (*Table 2*) [likelihood ratio test: $P < 0.001$, $NRI^{>50}$: 0.3%, $P = 0.724$; IDI: 0.4%, $P = 0.021$; Harrell's C baseline: 0.740 vs. Harrell's C IL-6: 0.741].

Table 1 Baseline characteristics and descriptive statistics for the whole cohort and for groups based on quartiles of interleukin-6

Variable	Total cohort	IL-6 Quartile 1	IL-6 Quartile 2	IL-6 Quartile 3	IL-6 Quartile 4	*P*-value
Patient count	2329	596	581	573	579	N/A
Demographics						
Male (%)	1716 (73.7%)	455 (76.3%)	416 (71.6%)	425 (74.2%)	421 (72.7%)	0.28
Race						
Caucasian	2303 (98.9%)	586 (98.3%)	575 (99.0%)	568 (99.1%)	574 (99.1%)	0.77
Other	26 (1.1%)	10 (1.7%)	6 (1.0%)	5 (0.8%)	5 (0.9%)	
Age (years)	68.8 (12.0)	65.7 (12.1)	69.0 (11.9)	69.7 (11.7)	70.9 (11.8)	<**0.001***
Clinical characteristics and co-morbidities						
Primary ischaemic HF aetiology	1126 (45.5%)	252 (43.2%)	256 (45.1%)	269 (47.6%)	262 (45.8%)	0.52
HF hospitalization in previous year	720 (30.9%)	180 (30.2%)	174 (29.9%)	186 (32.5%)	180 (31.1%)	0.79
Atrial fibrillation	1052 (45.2%)	216 (36.2%)	252 (43.4%)	289 (50.4%)	295 (50.9%)	<**0.001***
Diabetes mellitus	754 (32.4%)	155 (26.0%)	186 (32.0%)	210 (36.6%)	203 (35.1%)	<**0.001***
Hypertension	1455 (62.5%)	374 (62.8%)	356 (61.3%)	383 (66.8%)	342 (59.1%)	**0.048***
Anaemia	777 (36.6%)	115 (22.3%)	183 (35.6%)	219 (40.6%)	260 (47.0%)	<**0.001***
Smoking status						
None	852 (36.6%)	215 (36.1%)	216 (37.2%)	212 (37.0%)	209 (36.2%)	
Past	1140 (49.0%)	306 (51.3%)	275 (47.4%)	277 (48.3%)	282 (48.8%)	0.80
Current	335 (14.4%)	75 (12.6%)	89 (15.3%)	84 (14.7%)	87 (15.1%)	
NYHA functional class (prior to worsening HF)						
I	214 (9.2%)	58 (9.7%)	57 (9.8%)	54 (9.4%)	45 (7.8%)	
II	1075 (46.2%)	307 (51.5%)	278 (47.8%)	257 (44.9%)	233 (40.2%)	
III	666 (28.6%)	144 (24.2%)	171 (29.4%)	151 (26.4%)	200 (34.5%)	<**0.001***
IV	80 (3.4%)	17 (2.9%)	10 (1.7%)	28 (4.9%)	25 (4.3%)	
Physical examination						
BMI (kg/m^2)	27.8 (5.4)	27.7 (5.0)	27.8 (5.4)	28.1 (5.9)	27.5 (5.5)	0.36
Heart rate (b.p.m.)	80.0 (19.6)	75.2 (17.7)	79.6 (19.6)	81.1 (18.8)	84.3 (21.2)	<**0.001***
Systolic blood pressure (mmHg)	124.8 (22.0)	126.4 (20.0)	125.0 (20.9)	124.7 (22.4)	123.1 (24.5)	0.079
Diastolic blood pressure (mmHg)	75.0 (13.4)	76.8 (12.4)	75.2 (13.4)	74.8 (13.6)	73.1 (14.0)	<**0.001***
Successful completion of 6MWT	1469 (65.5%)	480 (82.1%)	385 (68.9%)	345 (63.8%)	259 (46.3%)	<**0.001***
6MWT distance	295.3 (130.9)	348.5 (117.3)	296.8 (128.5)	274.4 (125.6)	232.3 (127.6)	<**0.001***
Echocardiographic indices						
LVEF (%)	30.0 (25.0, 36.0)	30.0 (25.0, 35.0)	30.0 (25.0, 36.0)	30.0 (24.0, 38.0)	30.0 (25.0, 36.0)	0.999
LVEF > 40%	222 (10.6%)	33 (5.9%)	51 (9.9%)	64 (12.5%)	74 (14.7%)	<**0.001***
e' septal (cm/s)	6.6 (5.0, 9.4)	6.7 (5.0, 9.4)	6.0 (4.8, 9.1)	6.5 (5.0, 9.7)	7.0 (5.0, 9.7)	0.50
Left atrial diameter (mm)	47.5 (8.0)	46.9 (7.0)	47.3 (8.2)	47.8 (8.0)	47.9 (8.9)	0.21
Laboratory indices						
NT-proBNP (ng/mL)	2591.0 (1144.0, 5333.0)	1365.0 (587.4, 2917.0)	2162.5 (1046.5, 4422.5)	3300.0 (1513.0, 6764.0)	4602.0 (2330.0, 8876.0)	<**0.001***
IL-6 (pg/mL)	5.2 (2.8, 10.2)	1.9 (1.4, 2.4)	3.9 (3.3, 4.6)	7.1 (6.1, 8.3)	17.7 (13.1, 30.4)	<**0.001***

Table 1 Continued

Variable	Total cohort	IL-6 Quartile 1	Quartile 2	Quartile 3	Quartile 4	*P*-value
CRP (mg/L)	13.1 (5.8, 26.8)	4.8 (2.4, 10.4)	11.0 (6.0, 19.2)	16.8 (9.3, 29.5)	27.5 (15.7, 44.5)	<**0.001***
Troponin T (μg/mL)	0.0 (0.0, 0.1)	0.0 (0.0, 0.0)	0.0 (0.0, 0.0)	0.0 (0.0, 0.1)	0.0 (0.0, 0.1)	<**0.001***
Creatinine (μmol/mL)	100.8 (83.0, 129.0)	93.0 (78.0, 115.0)	100.0 (82.2, 128.0)	103.7 (86.7, 133.0)	108.0 (87.5, 145.0)	<**0.001***
eGFR (MDRD) (mL/min/1.73 m^2)	65.0 (26.0)	71.9 (25.8)	65.9 (27.3)	62.4 (24.7)	59.4 (24.7)	<**0.001***
Haemoglobin (g/dL)	13.2 (1.9)	13.8 (1.7)	13.3 (1.9)	13.0 (1.9)	12.7 (1.9)	<**0.001***
Mean corpuscular volume (fL)	90.5 (8.3)	90.8 (7.0)	90.8 (7.6)	90.1 (9.5)	90.3 (9.1)	0.48
Iron (mg/dL)	8.0 (5.0, 13.0)	12.0 (8.0, 16.0)	9.0 (6.0, 13.0)	7.0 (5.0, 11.0)	6.0 (4.0, 9.0)	<**0.001***
Ferritin (μg/L)	102.0 (49.0, 193.0)	111.0 (49.0, 196.0)	92.0 (45.0, 185.0)	101.0 (48.0, 192.5)	105.0 (54.0, 205.0)	0.057
Ferritin < 20 μg/L	143 (6.4%)	39 (6.8%)	34 (6.2%)	42 (7.7%)	28 (5.1%)	0.37
Transferrin (g/L)	2.1 (0.7)	2.1 (0.6)	2.1 (0.7)	2.1 (0.7)	2.0 (0.7)	<**0.001***
Transferrin saturation (%)	17.1 (10.9, 24.9)	23.5 (17.1, 30.2)	18.4 (12.8, 25.5)	15.0 (9.9, 21.9)	11.9 (8.4, 18.1)	<**0.001***
Hepcidin (nmol/L)	6.3 (2.2, 16.4)	6.6 (2.7, 14.0)	5.3 (2.1, 11.8)	5.6 (1.7, 15.9)	8.4 (2.3, 23.9)	<**0.001***
Medications at baseline						
BB (target dose)	125 (5.4%)	36 (6.0%)	36 (6.2%)	30 (5.2%)	23 (4.0%)	0.31
BB (% target dose)	0.3 (0.1, 0.5)	0.3 (0.1, 0.5)	0.3 (0.1, 0.5)	0.2 (0.1, 0.5)	0.2 (0.0, 0.4)	**0.008***
ACEi/ARB (target dose)	299 (12.8%)	104 (17.4%)	81 (13.9%)	63 (11.0%)	51 (8.8%)	<**0.001***
ACEi/ARB (% target dose)	0.3 (0.0, 0.5)	0.3 (0.1, 0.5)	0.3 (0.0, 0.5)	0.3 (0.0, 0.5)	0.3 (0.0, 0.5)	<**0.001***
MRA	1239 (53.2%)	342 (57.4%)	308 (53.0%)	311 (54.3%)	278 (48.0%)	**0.013***
Diuretic	2327 (99.9%)	595 (99.8%)	581 (100.0%)	572 (99.8%)	579 (100.0%)	0.57
Loop diuretic	2317 (99.5%)	594 (99.7%)	577 (99.3%)	570 (99.5%)	576 (99.5%)	0.87
Digoxin	437 (18.8%)	91 (15.3%)	109 (18.8%)	102 (17.8%)	135 (23.3%)	**0.005***
Oral anti-diabetic	470 (62.3%)	105 (67.7%)	117 (62.9%)	129 (61.4%)	119 (58.6%)	0.36

6MWT, 6-min walk test; ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; BB, β-adrenoreceptor blocker; BMI, body mass index; BNP, brain natriuretic peptide; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease formula); HF, heart failure; IL-6, interleukin-6; LVEF, left ventricular ejection fraction; MRA, mineralocorticoid receptor antagonist; NT-proBNP, N-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association.

* $P \leq 0.05$.

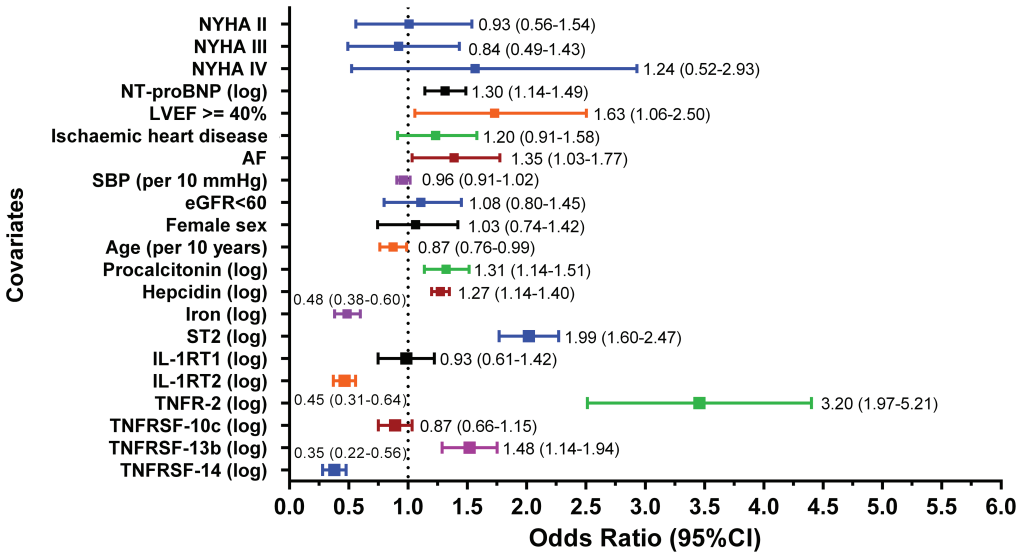

Figure 1 Forest plot with a multivariable logistic regression model for interleukin (IL)-6 levels above or equal to the 95th percentile of normal values (≥ 4.45 pg/mL). For New York Heart Association (NYHA) functional class, class I is used as a reference category. Odds ratios and 95% confidence intervals (CI) are presented next to each variable. The following variables were independent predictors of higher IL-6 levels: younger age ($P = 0.032$), the logarithms of N-terminal pro-brain natriuretic peptide (NT-proBNP), procalcitonin, hepcidin, ST2, IL-1 receptor type 2 (IL-1RT2), tumour necrosis factor receptor 2 (TNFR-2), tumour necrosis factor receptor superfamily member 14 (TNFRSF-14) and lower iron concentrations ($P < 0.001$ for all), the logarithm of tumour necrosis factor receptor superfamily member 13b (TNFRSF-13b) ($P = 0.004$) as well as having atrial fibrillation (AF) ($P = 0.028$) and heart failure with preserved ejection fraction ($P = 0.027$). eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease formula); LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; IL-1RT1, interleukin-1 receptor type 1; TNFRSF-10c, tumour necrosis factor receptor superfamily member 10c.

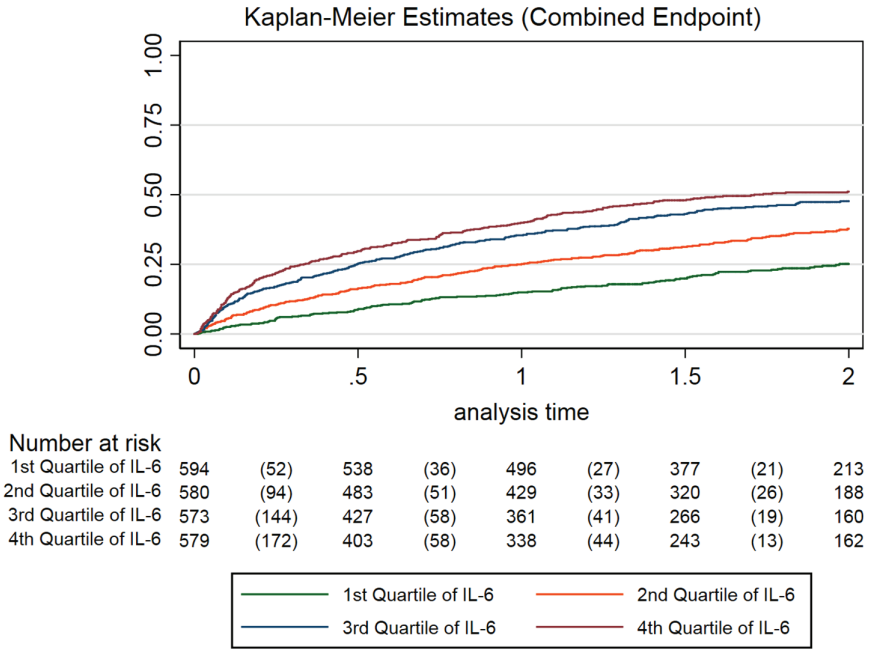

Figure 2 Kaplan–Meier plot displaying the time to event (combined endpoint) curves for patients in different quartiles of interleukin (IL)-6 levels (the 1st quartile contains the lowest values). The log-rank test was significant ($P < 0.001$).

Table 2 Cox regression models for the prediction of the combined outcome and all-cause mortality and competing risk regression models for the prediction of heart failure hospitalization, cardiovascular and non-cardiovascular mortality by each doubling of interleukin-6 [log_2(IL-6)]

Model	Combined endpoint		All-cause mortality		Cardiovascular mortality		Non-cardiovascular mortality		HF hospitalization	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
a	1.25 (1.20–1.30)	<0.001	1.31 (1.25–1.38)	<0.001	1.26 (1.19–1.34)	<0.001	1.37 (1.24–1.50)	<0.001	1.06 (0.99–1.13)	0.069
b	1.19 (1.14–1.23)	<0.001	1.23 (1.17–1.30)	<0.001	1.18 (1.11–1.25)	<0.001	1.33 (1.19–1.47)	<0.001	1.03 (0.96–1.10)	0.388
c	1.16 (1.12–1.21)	<0.001	1.22 (1.16–1.29)	<0.001	1.17 (1.09–1.24)	<0.001	1.32 (1.19–1.47)	<0.001	1.01 (0.94–1.09)	0.732
d	1.16 (1.11–1.21)	<0.001	1.22 (1.16–1.29)	<0.001	1.16 (1.09–1.24)	<0.001	1.31 (1.18–1.45)	<0.001	1.01 (0.94–1.08)	0.833
e	1.08 (1.03–1.13)	0.001	1.14 (1.07–1.20)	<0.001	N/A		N/A		N/A	

BMI, body mass index; CI, confidence interval; eGFR, estimated glomerular filtration rate (Modification of Diet in Renal Disease formula); HR, hazard ratio; IDI, integrated discrimination improvement; IL-6 interleukin-6; NRI, net reclassification index.
Model a: IL-6 as a univariable predictor.
Model b: model a with the addition of age, sex, BMI and eGFR.
Model c: model b with the addition of co-morbidities (smoking status, alcohol consumption, diabetes mellitus, eGFR < 60 mL/min/1.73 m^2 and history of any of the following: atrial fibrillation, myocardial infarction, stroke, peripheral arterial disease, chronic obstructive pulmonary disease and hypertension.
Model d: model c with the addition of heart failure medication (angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, β-adrenoreceptor blockers, mineralocorticoid receptor antagonists, digoxin, diuretics and loop diuretics).
Model e: the addition of IL-6 to the proportional hazards model for the prediction of the combined outcome already published by our group.[16]
For model e, although model fit based on the likelihood ratio test was better for both the combined endpoint and all-cause mortality ($P = 0.002$ and $P < 0.001$, respectively), reclassification indices were not significantly improved [combined endpoint: $NRI^{>50}$: −0.4%, $P = 0.610$; IDI: 0.2%, $P = 0.031$; Harrell's C baseline: 0.713 vs. Harrell's C log_2(IL-6): 0.715; all-cause mortality: $NRI^{>50}$: 0.3%, $P = 0.724$; IDI: 0.4%, $P = 0.021$; Harrell's C baseline: 0.740 vs. Harrell's C log_2(IL-6): 0.741].

Discussion

In this retrospective study of a large and heterogeneous cohort of HF patients, approximately half of all patients had abnormally elevated IL-6 levels based on previously defined normal values. Having HFpEF and AF as well as younger age, decreasing iron values and increasing NT-proBNP, PCT, hepcidin and TNF-α/IL-1 related biomarker values were independent predictors of higher IL-6 levels. IL-6 also independently predicted a combined endpoint of all-cause mortality and hospitalization and all-cause as well as cause-specific mortality individually, but the addition of IL-6 to previous predictive models for this cohort did not improve risk stratification.

In our study, having HFpEF was a strong independent predictor of elevated IL-6 levels. These findings are in line with a previous study, which demonstrated that IL-6 and TNF-α significantly correlated with echocardiographic indices of diastolic dysfunction and were both shown to downregulate the expression of sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2) channels in cardiomyocytes.[18] SERCA2 is involved in diastolic cardiomyocyte relaxation by mediating calcium reabsorption in the sarcoplasmic reticulum.[18] Additionally, IL-6 increases cardiomyocyte stiffness by reducing titin phosphorylation.[19] These processes might in turn explain the association of IL-6 with diastolic dysfunction. Having AF was also an independent predictor of higher IL-6, which is in agreement with previous studies that support a general involvement of inflammatory processes in the pathophysiology of AF.[20] In contrast to previous studies that have identified elevated IL-6 levels in older patients,[6] there was an independent association between higher IL-6 and younger age in this cohort. This could perhaps be explained by alterations in IL-6 trans-signaling that occur with ageing and lead to reduction of circulating soluble glycoprotein 130, a soluble receptor which acts as an inhibitor of IL-6 function.[21] As such, IL-6 might be lower in older individuals with HF because its biological actions are achieved using smaller concentrations. However, since no additional information was available regarding plasma glycoprotein 130 levels or other IL-6 signalling components, this hypothesis could not be investigated.

Patients with higher levels of IL-6 had a higher prevalence of anaemia and disturbed indices of iron metabolism, and lower iron levels were an independent predictor of elevated IL-6 levels. IL-6 signalling activates the acute phase response in the liver and hepcidin is an acute phase protein produced as a result.[4,22] Hepcidin controls systemic iron metabolism and causes hypoferremia, which is mediated by IL-6.[23] IL-6 also induces a significant increase in the expression of hepcidin mRNA, independent of IL-1 or TNF-α activity.[22] Anaemia plays an important role in HF as it has been shown to be associated with a poor prognosis and can affect exercise capacity, the development of depression and potentially the myocardium directly.[24] Previous studies in chronic HF found no association between IL-6 and hepcidin levels but were limited in sample size.[24] Our data, taken together with previous studies, suggest that IL-6 signalling is an important biological pathway leading to anaemia and/or iron deficiency in HF and might thus warrant future investigation as a potential treatment target for modulating these pathologic processes.

NT-proBNP was also a significant independent predictor of elevated IL-6 levels. NT-proBNP is produced in response to cardiac stretch,[25] and experimental evidence has indeed demonstrated that stretched cardiomyocytes and cardiac fibroblasts elaborate TNF-α/IL-6 and IL-1β, respectively.[19] This is also in agreement with the finding that elevated IL-6 levels are independently predicted by higher TNF-α/IL-1 related biomarkers. PCT was an

additional independent predictor of elevated IL-6 levels. PCT is the prohormone of calcitonin, it is mainly produced by the liver and has been shown to behave as an acute phase protein, inducible by both TNF-α and IL-6; its primary biological action is similar to mature calcitonin and involves the reduction of blood calcium levels.[26] In addition, PCT acts as a chemokine at sites of injury and has been shown to induce inflammatory cytokine production in macrophages, the principal immune cells that produce IL-6.[27] Current opinion supports an association of elevated PCT levels with infectious processes. However, up to a third of patients with chronic kidney disease may have abnormally elevated PCT, which resolves after the initiation of renal replacement therapy.[28] This suggests an additional role of PCT in chronic inflammatory conditions, which might by extension also apply to HF.

Previous studies have demonstrated that IL-6 significantly predicted mortality in acute HF and acute coronary syndromes as well as chronic HF, although in relatively small cohorts (75 and 102 patients, respectively).[13,14] In another study, elevated plasma IL-6 was associated with an increased risk of death and higher urine IL-6 levels were associated with a higher risk of having eGFR < 60 mL/min/1.73 m^2.[7] To our knowledge, our study is the first to incorporate IL-6 in a validated risk prediction model for all-cause mortality and hospitalization in a multi-national and diverse HF population, with an adequate sample size. We demonstrated that each doubling of IL-6 independently predicted HF hospitalization and all-cause as well as cause-specific mortality. We also demonstrate that plasma IL-6 proportionally increases as eGFR decreases. However, IL-6 did not improve previously published risk models for this cohort.

Clinical implications of interleukin-6 levels in heart failure

Firstly, having HFpEF, AF, iron deficiency and increased NT-proBNP, PCT, hepcidin and TNF-α/IL-1 related biomarker levels were independent predictors of higher IL-6 levels and IL-6 was associated with various indices of iron metabolism. As mentioned previously specifically for iron deficiency, previous basic studies have demonstrated that the effects of IL-6 are independent of TNF-α/IL-1.[22] The latter, together with a study demonstrating that longstanding TNF-α blockade in rats with HF leads to reactive elevation of plasma IL-6,[29] might constitute a potential explanation as to why TNF-α blockade has thus far failed to demonstrate beneficial effects in patients with HF. Although our findings do not permit for causal inferences to be drawn, they do warrant further investigation of any potential pharmaceutical applications of IL-6 signalling modulation in future studies. Lastly, IL-6 was an independent predictor of adverse events, although it did not improve discrimination in previously published risk models for this cohort. However, it should be noted that these models included haemoglobin as well as NT-proBNP, which may both change in tandem with IL-6 levels and may thus account for the variance explained by IL-6. In this study, we identified a strong relationship between IL-6 and indices of iron metabolism, which could lead to incorrect deductions due to multicollinearity.

Limitations

In this retrospective study, we only investigated the associations of IL-6 plasma levels with clinical characteristics, other inflammatory biomarkers and outcomes. No data were currently available on genetic expression parameters or other proteomic markers of IL-6 signalling. As a result, other signalling components such as glycoprotein 130 and the soluble IL-6 receptor were not investigated next to plasma IL-6. Furthermore, we did not directly evaluate TNF-α/IL-1 themselves in relation to IL-6 and no longitudinal data on IL-6 levels were available. Additionally, no data on the prevalence of autoimmune disease in this cohort were available. Finally, although numerous associations were identified between IL-6 and clinical measurements/outcomes, these require further individual, dedicated investigation in future studies.

Conclusion

In a large cohort of HF patients, approximately half had IL-6 levels above the 95th percentile of normal values. Independent predictors of IL-6 were the presence of younger age, HFpEF, AF, iron deficiency and higher NT-proBNP, PCT, hepcidin and TNF-α/IL-1 related biomarker levels. Finally, plasma levels of IL-6 independently predicted death and/or HF hospitalization but did not improve discrimination in previous models. These findings suggest an important, albeit limited, role for IL-6 as an adjunct for risk stratification in HF and constitute preliminary evidence that warrants further investigation of any potential pharmaceutical applications of IL-6 signalling modulation and especially in specific target groups, such as patients with elevated IL-6 or patients with HFpEF, AF, or iron deficiency. Nevertheless, these results should be individually validated in future studies that can support causal inferences.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Differences in baseline characteristics and descriptive statistics for patients with and without available interleukin-6 measurements.

Figure S1. Forest plot with subgroup analyses per relevant covariate for the prediction of the combined endpoint (all-cause mortality and heart failure hospitalization) by interleukin-6. No significant statistical interactions were identified.

Figure S2. Cumulative incidence function plot displaying the time to event (cardiovascular death) curves for patients in different quartiles of interleukin-6 levels (the 1st quartile contains the lowest values).

Figure S3. Cumulative incidence function plot displaying the time to event (non-cardiovascular death) curves for patients in different quartiles of interleukin-6 levels (the 1st quartile contains the lowest values).

Figure S4. Cumulative incidence function plot displaying the time to event (heart failure rehospitalization) curves for patients in different quartiles of interleukin-6 levels (the 1st quartile contains the lowest values).

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References

1. Shirazi LF, Bissett J, Romeo F, Mehta JL. Role of inflammation in heart failure. *Curr Atheroscler Rep* 2017;**19**:27.
2. Mann DL, McMurray JJ, Packer M, Swedberg K, Borer JS, Colucci WS, Djian J, Drexler H, Feldman A, Kober L, Krum H, Liu P, Nieminen M, Tavazzi L, van Veldhuisen DJ, Waldenstrom A, Warren M, Westheim A, Zannad F, Fleming T. Targeted anticytokine therapy in patients with chronic heart failure: results of the Randomized Etanercept Worldwide Evaluation (RENEWAL). *Circulation* 2004;**109**:1594–1602.
3. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJ, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PR, Troquay RP, Libby P, Glynn RJ; CANTOS Trial Group. Antiinflammatory therapy with canakinumab for atherosclerotic disease. *N Engl J Med* 2017;**377**:1119–1131.
4. Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. *Int J Biol Sci* 2012;**8**:1237–1247.
5. IL6R Genetics Consortium Emerging Risk Factors Collaboration. Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies. *Lancet* 2012;**379**:1205–1213.
6. Maggio M, Guralnik JM, Longo DL, Ferrucci L. Interleukin-6 in aging and chronic disease: a magnificent pathway. *J Gerontol A Biol Sci Med Sci* 2006;**61**:575–584.
7. Jackson K, Rao V, Hanberg J, Mahoney D, Chunara Z, Thomas D, Hodson D, Tarleton C, Chen M, Jacoby D, Riello R, Ahmad T, Testani J. Inflammation and cardio-renal interactions in heart failure: a potential role for interleukin-6. *Eur J Heart Fail* 2018;**20**:933–934.
8. Marcus GM, Whooley MA, Glidden DV, Pawlikowska L, Zaroff JG, Olgin JE. Interleukin-6 and atrial fibrillation in patients with coronary artery disease: data from the Heart and Soul Study. *Am Heart J* 2008;**155**:303–309.
9. Plenz G, Song ZF, Tjan TD, Koenig C, Baba HA, Erren M, Flesch M, Wichter T, Scheld HH, Deng MC. Activation of the cardiac interleukin-6 system in advanced heart failure. *Eur J Heart Fail* 2001;**3**:415–421.
10. Bielecka-Dabrowa A, Sakowicz A, Pietrucha T, Misztal M, Chruściel P, Rysz J, Banach M. The profile of selected single nucleotide polymorphisms in patients with hypertension and heart failure with preserved and mid-range ejection fraction. *Sci Rep* 2017;**7**:8974.
11. Gwechenberger M, Hulsmann M, Berger R, Graf S, Springer C, Stanek B, Pacher R. Interleukin-6 and B-type natriuretic peptide are independent predictors for worsening of heart failure in patients with progressive congestive heart failure. *J Heart Lung Transplant* 2004;**23**:839–844.
12. Eskandari V, Amirzargar AA, Mahmoudi MJ, Rahnemoon Z, Rahmani F, Sadati S, Rahmati Z, Gorzin F, Hedayat M, Rezaei N. Gene expression and levels of IL-6 and TNFα in PBMCs correlate with severity and functional class in patients with chronic heart failure. *Ir J Med Sci* 2018;**187**:359–368.
13. Maeda K, Tsutamoto T, Wada A, Mabuchi N, Hayashi M, Tsutsui T, Ohnishi M, Sawaki M, Fujii M, Matsumoto T, Kinoshita M. High levels of plasma brain natriuretic peptide and interleukin-6 after optimized treatment for heart failure are independent risk factors for morbidity and mortality in patients with congestive heart failure. *J Am Coll Cardiol* 2000;**36**:1587–1593.
14. Hamzic-Mehmedbasic A. Inflammatory cytokines as risk factors for mortality after acute cardiac events. *Med Arch* 2016;**70**:252–255.
15. Garbers C, Heink S, Korn T, Rose-John S. Interleukin-6: designing specific therapeutics for a complex cytokine. *Nat Rev Drug Discov* 2018;**17**:395–412.
16. Voors AA, Ouwerkerk W, Zannad F, van Veldhuisen DJ, Samani NJ, Ponikowski P, Ng LL, Metra M, ter Maaten JM, Lang CC, Hillege HL, van der Harst P, Filippatos G, Dickstein K, Cleland JG, Anker SD, Zwinderman AH. Development and validation of multivariable models to predict mortality and hospitalization in patients with heart failure. *Eur J Heart Fail* 2017;**19**:627–634.
17. Todd J, Simpson P, Estis J, Torres V, Wub AH. Reference range and short- and long-term biological variation of interleukin (IL)-6, IL-17A and tissue necrosis factor-alpha using high sensitivity assays. *Cytokine* 2013;**64**:660–665.
18. Wu CK, Lee JK, Chiang FT, Yang CH, Huang SW, Hwang JJ, Lin JL, Tseng CD, Chen JJ, Tsai CT. Plasma levels of tumor necrosis factor-α and interleukin-6 are associated with diastolic heart failure through downregulation of sarcoplasmic reticulum Ca^{2+} ATPase. *Crit Care Med* 2011;**39**:984–992.
19. Van Linthout S, Tschöpe C. Inflammation – cause or consequence of heart failure or both? *Curr Heart Fail Rep* 2017;**14**:251–265.
20. Hu YF, Chen YJ, Lin YJ, Chen SA. Inflammation and the pathogenesis of atrial fibrillation. *Nat Rev Cardiol* 2015;**12**:230–243.
21. Ticinesi A, Meschi T, Lauretani F, Felis G, Franchi F, Pedrolli C, Barichella M, Benati G, Di Nuzzo S, Ceda GP, Maggio M. Nutrition and inflammation in older individuals: focus on vitamin D, n-3 polyunsaturated fatty acids and whey proteins. *Nutrients* 2016;**8**:186.
22. Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood* 2003;**101**:2461–2463.
23. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, Ganz T. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 2004;**113**:1271–1276.
24. Jankowska EA, Von Haehling S, Anker SD, MacDougall IC, Ponikowski P. Iron deficiency and heart failure: diagnostic dilemmas and therapeutic perspectives. *Eur Heart J* 2013;**34**:816–829.
25. George M, Jena A, Srivatsan V, Muthukumar R, Dhandapani V. GDF 15 – a novel biomarker in the offing for heart failure. *Curr Cardiol Rev* 2016;**12**:37–46.
26. Nijsten MW, Olinga P, The TH, de Vries EG, Koops HS, Groothuis GM, Limburg PC, ten Duis HJ, Moshage H, Hoekstra HJ, Bijzet J, Zwaveling JH. Procalcitonin behaves as a fast responding acute phase protein in vivo and in vitro. *Crit Care Med* 2000;**28**:458–461.
27. Becker KL, Nylén ES, White JC, Müller B, Snider RH. Procalcitonin and the calcitonin gene family of peptides in inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. *J Clin Endocrinol Metab* 2004;**89**:1512–1525.
28. Grace E, Turner RM. Use of procalcitonin in patients with various degrees of chronic kidney disease including renal replacement therapy. *Clin Infect Dis* 2014;**59**:1761–1767.
29. Haugen E, Täng MS, Isic A, Andersson B, Fu M. TNFalpha antagonist upregulates interleukin-6 in rats with hypertensive heart failure. *Int J Cardiol* 2008;**130**:64–68.